Semi-generic analysis of Kraken results from Sunbeam

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Required input:

- Kraken2 output from Sunbeam (`example_kraken_all_samples.tsv`)
- Sample metadata (could also construct it from the sample names in the Kraken dataset)

Analyses we will perform:

- Alpha diversity
- Barplots of microbial composition
- Beta diversity AKA PCoA
- DESeq2 to identify differential taxa

Search the document for "CHANGEME" to see which lines of code will require project-specific tweaks.

Load libraries

```
library(tidyverse)
library(phyloseq)
library(DESeq2)

# speedyseq is just to speed up tax_glom
# remotes::install_github("mikemc/speedyseq")
library(speedyseq)

theme_set(theme_bw(base_size=15))
```

Import data

```
# CHANGEME: change filepath to your input data
# N.B. You might need to delete the "#" on the second line (right before "OTU ID")
# in your input file. Do this in any text editor, like vi.
kraken.df <- read.table("example_kraken_all_samples.tsv",
                        sep="\t", quote="", header=T)

# remove "-taxa" suffix
colnames(kraken.df) <- str_replace(colnames(kraken.df), ".taxa$", "")

# subset to just data columns
kraken.data.df <- kraken.df %>%
  column_to_rownames("OTU.ID") %>%
  dplyr::select(-Consensus.Lineage)
```

```
dim(kraken.data.df)
```

```
## [1] 6565   16
```

```
head(kraken.data.df)
```

```
##         CR40.2002_044w CR40.2004_044w CR40.2005_044w CR40.2006_044w
## 2                 7953          18066          22056          10786
## 1239              4224          15247           5666           4349
## 186801             154            564            143            157
## 186802            6016          21858           5837           6674
## 186803            2582          10317           2453           2407
## 1506553            129            733            130            188
##         CR40.2007_044w CR40.2008_044w CR40.2010_044w AL.0004_044w AL.0005_044w
## 2                23040          15772          27846        11038         6493
## 1239             23822          11088           8162         3296         6130
## 186801             888            373            216          133          207
## 186802           35103          16897          11008         4258        10365
## 186803           14085           6776           4081         1695         4082
## 1506553            796            494            234           90          225
##         AL.0006_044w AL.0007_044w AL.0008_044w AL.0010_044w AL.0012_044w
## 2               2588        13389         7739        15706         8642
## 1239            1941        13640        10183        13891        11088
## 186801            73          462          262          508          365
## 186802          2796        21574        14860        21257        18023
## 186803          1386         7990         5573        10053         8115
## 1506553           56          523          519          504          514
##         AL.0014_044w AL.0015_044w
## 2              14922        23453
## 1239           13761         8700
## 186801           651          295
## 186802         27305        10149
## 186803         10761         3840
## 1506553          601          220
```

- 6565 taxa, 16 samples
- Make sure these numbers (especially the number of samples) make sense

Import sample metadata

```
# CHANGEME: you can either create sample metadata from the sample names
# or import the metadata from a different file
meta.df <- data.frame(
  sample.ID=colnames(kraken.data.df))

# CHANGEME: create new columns based on sample name
meta.df <- meta.df %>%
  tidyr::separate(sample.ID, c("diet.mouseID", "age.weeks"), sep="_", remove=F) %>%
  tidyr::separate(diet.mouseID, c("diet", "mouseID"), sep="\\.") %>%
  column_to_rownames("sample.ID")

# CHANGEME: convert certain columns to factors in order to specify their order in plots
meta.df <- meta.df %>%
  mutate(diet.fctr=factor(diet, levels=c("AL", "CR40")))
```

Convert `Consensus Lineage` column into a taxonomy df

```
kraken.tax.df <- data.frame(str_split_fixed(kraken.df$Consensus.Lineage, "__|; ", n=14)[, seq(2,14,2)])
colnames(kraken.tax.df) <- c("kingdom", "phylum", "class", "order", "family", "genus", "species")
rownames(kraken.tax.df) <- kraken.df$OTU.ID
```

Create phyloseq object

```
physeq <- phyloseq(
  kraken.data.df %>% as.matrix %>% otu_table(taxa_are_rows=T),
  meta.df %>% sample_data,
  kraken.tax.df %>% as.matrix %>% tax_table)
```

Subset to bacteria

```
physeq.bac <- physeq %>% subset_taxa(kingdom=="Bacteria")
```

- 6242 bacterial taxa

Percent of reads assigned to each taxonomy level

```
physeq.bac.tax.df.w.tax.sum <- data.frame(taxa.sum=taxa_sums(physeq.bac)) %>%
  merge(data.frame(tax_table(physeq.bac)), by="row.names")

1 - sum(physeq.bac.tax.df.w.tax.sum$taxa.sum[physeq.bac.tax.df.w.tax.sum$family == ""]) / sum(physeq.bac
```

```
## [1] 0.9491975
```

```
1 - sum(physeq.bac.tax.df.w.tax.sum$taxa.sum[physeq.bac.tax.df.w.tax.sum$genus == ""]) / sum(physeq.bac
```

```
## [1] 0.9268306
```

```
1 - sum(physeq.bac.tax.df.w.tax.sum$taxa.sum[physeq.bac.tax.df.w.tax.sum$species == ""]) / sum(physeq.ba
```

```
## [1] 0.8740184
```

- These numbers describe what percent of reads could be mapped to a particular taxonomy level
- For example, 92.7% of reads could be mapped at least to the genus level

Aggregate to different taxonomy levels (slow)

```
physeq.bac.phylum <- physeq.bac %>% tax_glom(taxrank="phylum")
physeq.bac.class <- physeq.bac %>% tax_glom(taxrank="class")
physeq.bac.family <- physeq.bac %>% tax_glom(taxrank="family")
physeq.bac.genus <- physeq.bac %>% tax_glom(taxrank="genus")
physeq.bac.species <- physeq.bac %>% tax_glom(taxrank="species")
```

- Aggregation to the species level is slowest

```
ntaxa(physeq.bac.phylum)
```

```
## [1] 37
```

```
ntaxa(physeq.bac.class)
```

```
## [1] 73
```

```
ntaxa(physeq.bac.family)
```

```
## [1] 384
```

```
ntaxa(physeq.bac.genus)
```

```
## [1] 1463
```

```
ntaxa(physeq.bac.species)
```

```
## [1] 5487
```

- This tells you how many unique bacterial phyla, families, genera, etc. were detected

Add genus_and_species annotation to physeq.bac.species for plotting purposes

```
tax_table(physeq.bac.species) <- data.frame(tax_table(physeq.bac.species)) %>%
  mutate(genus_and_species=str_c(genus, species, sep=" ")) %>%
  as.matrix %>% tax_table
```

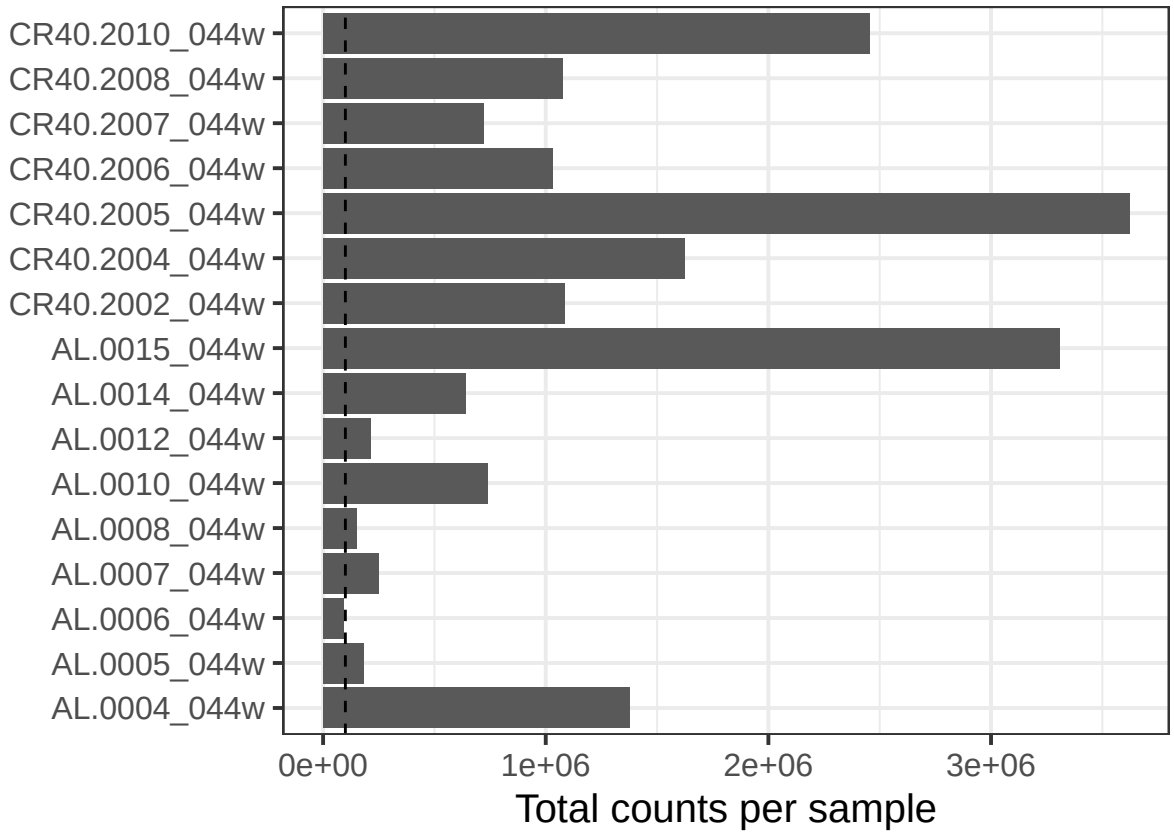
Optional: Filtration

Samples

It may be helpful to discard samples with very few reads and taxons that were observed very infrequently. The defaults provided here are to include samples with at least 10k counts, and taxons with at least 0.01% relative abundance in 20% of samples.

Here, I perform filtering for just the genus phyloseq object, but could do the same thing for the phyloseq objects at other taxonomy levels.

```
# CHANGEME: update filtering parameters as desired
MIN.COUNTS.PER.SAMPLE <- 1e5

data.frame(sample_sums(physeq.bac)) %>%
  setNames("sample.sum") %>%
  rownames_to_column("sample.ID") %>%
  ggplot(aes(x=sample.sum, y=sample.ID)) +
  geom_bar(stat="identity") +
  geom_vline(xintercept=MIN.COUNTS.PER.SAMPLE, lty=2) +
  labs(x="Total counts per sample", y="")
```



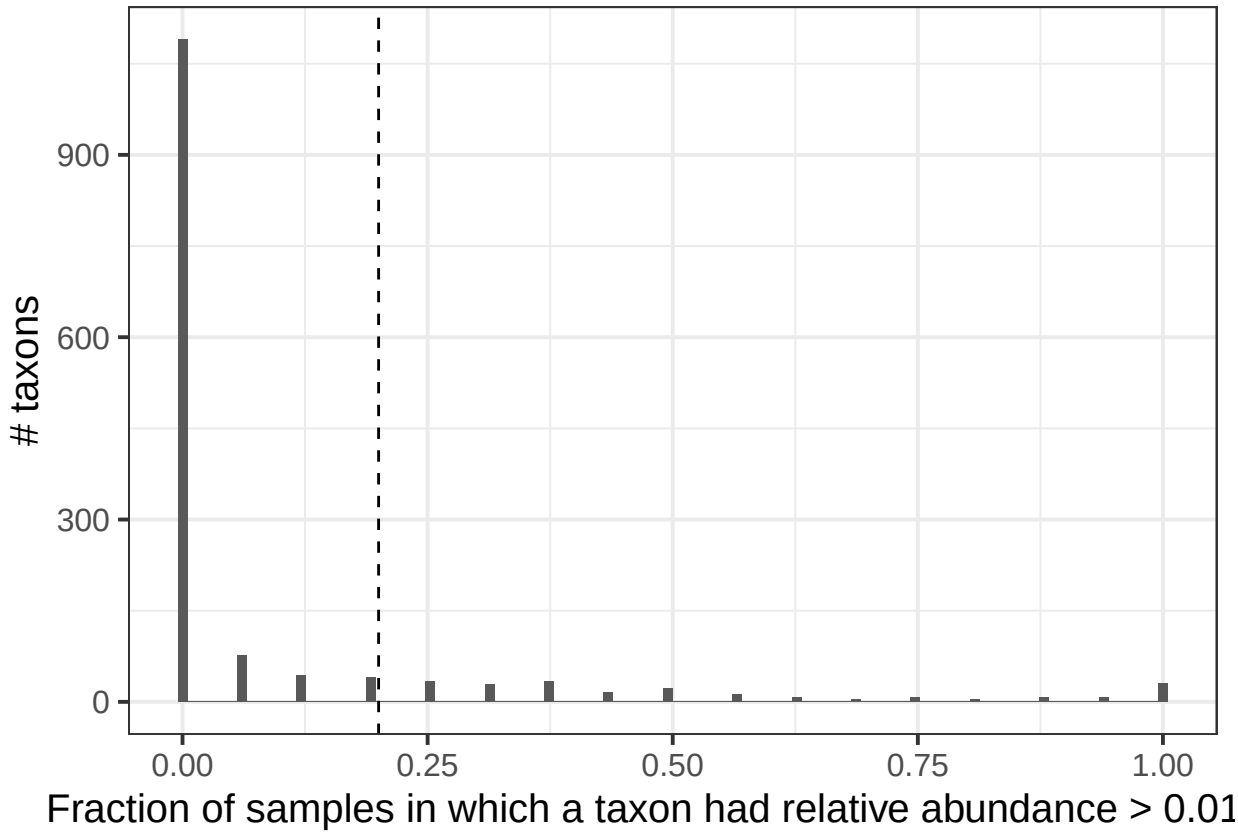

```
samples.to.keep.bool <- sample_sums(physeq.bac) > MIN.COUNTS.PER.SAMPLE

# CHANGEME: apply filtering for all phyloseq objects that you want
physeq.bac.genus.filt <- physeq.bac.genus %>%
  subset_samples(samples.to.keep.bool)
physeq.bac.species.filt <- physeq.bac.species %>%
  subset_samples(samples.to.keep.bool)
```

Taxa

Can filter the phyloseq objects at other taxonomy levels too.

Genus

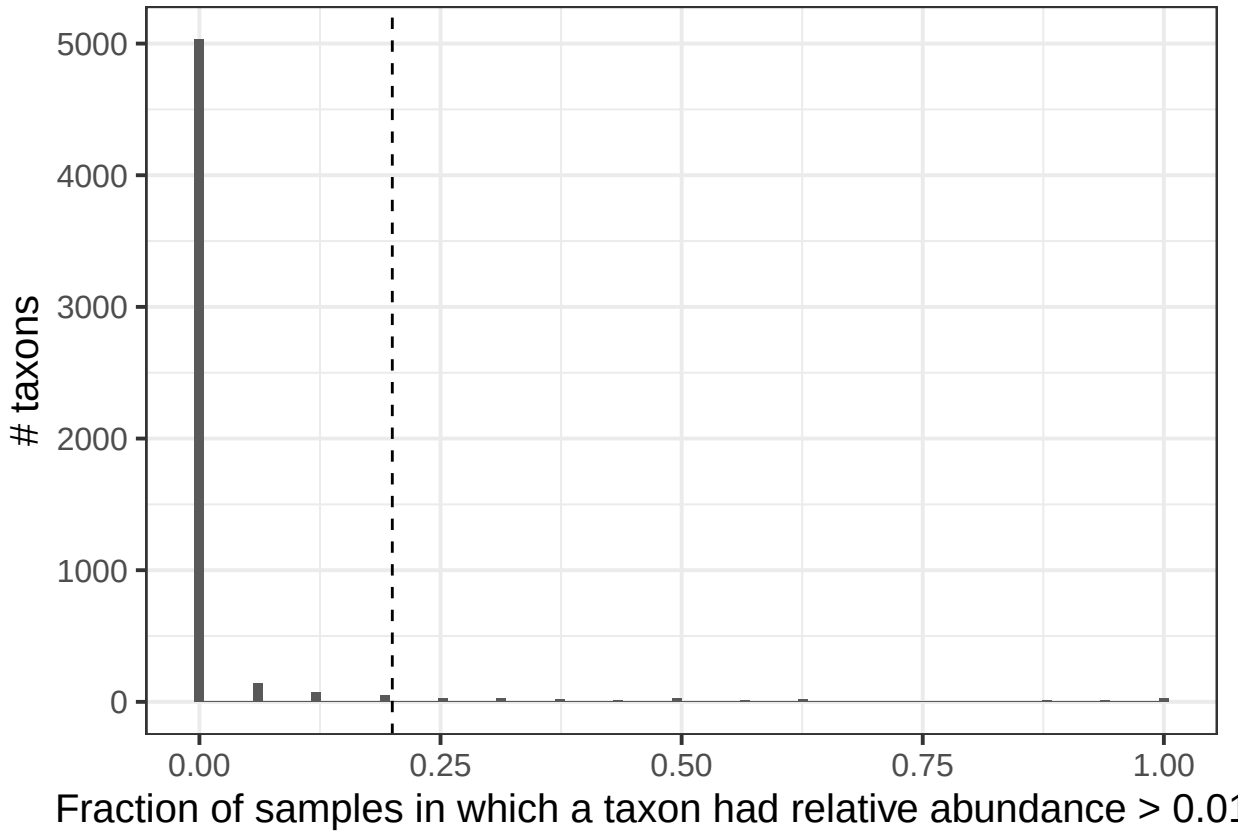
```
# CHANGEME: update filtering parameters as desired
MIN.REL.ABUND <- 1e-4
MIN.PERC.SAMPLES.W.TAXON.AT.REL.ABUND <- 0.2

bac.genus.rel.abund.mat <- physeq.bac.genus %>%
  transform_sample_counts(function(OTU) OTU/sum(OTU)) %>%
  otu_table %>% as.matrix
data.frame(
  frac.taxon.w.sufficient.rel.abund=rowSums(bac.genus.rel.abund.mat > MIN.REL.ABUND) / ncol(bac.genus.r
  ggplot(aes(frac.taxon.w.sufficient.rel.abund)) +
  geom_histogram(bins=100) +
  geom_vline(xintercept=MIN.PERC.SAMPLES.W.TAXON.AT.REL.ABUND, lty=2) +
  labs(y="# taxons", x=sprintf("Fraction of samples in which a taxon had relative abundance > %.3f%%", M
```




```
genera.to.keep.bool <- rowSums(bac.genus.rel.abund.mat > MIN.REL.ABUND) / ncol(bac.genus.rel.abund.mat)
physeq.bac.genus.filt <- physeq.bac.genus.filt %>%
  subset_taxa(genera.to.keep.bool)
```

Species

```
# CHANGEME: update filtering parameters as desired
MIN.REL.ABUND <- 1e-4
MIN.PERC.SAMPLES.W.TAXON.AT.REL.ABUND <- 0.2

bac.species.rel.abund.mat <- physeq.bac.species %>%
  transform_sample_counts(function(OTU) OTU/sum(OTU)) %>%
  otu_table %>% as.matrix
data.frame(
  frac.taxon.w.sufficient.rel.abund=rowSums(bac.species.rel.abund.mat > MIN.REL.ABUND) / ncol(bac.specie
  ggplot(aes(frac.taxon.w.sufficient.rel.abund)) +
  geom_histogram(bins=100) +
  geom_vline(xintercept=MIN.PERC.SAMPLES.W.TAXON.AT.REL.ABUND, lty=2) +
  labs(y="# taxons", x=sprintf("Fraction of samples in which a taxon had relative abundance > %.3f%%", M
```




```
species.to.keep.bool <- rowSums(bac.species.rel.abund.mat > MIN.REL.ABUND) / ncol(bac.species.rel.abund
physeq.bac.species.filt <- physeq.bac.species.filt %>%
  subset_taxa(species.to.keep.bool)
```

Summary of filtration

```
physeq.bac.genus
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:          [ 1463 taxa and 16 samples ]:
## sample_data() Sample Data:        [ 16 samples by 4 sample variables ]:
## tax_table()   Taxonomy Table:     [ 1463 taxa by 7 taxonomic ranks ]:
## taxa are rows
```

```
physeq.bac.genus.filt
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:          [ 213 taxa and 15 samples ]:
## sample_data() Sample Data:        [ 15 samples by 4 sample variables ]:
## tax_table()   Taxonomy Table:     [ 213 taxa by 7 taxonomic ranks ]:
## taxa are rows
```

```
physeq.bac.species
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:          [ 5487 taxa and 16 samples ]:
## sample_data() Sample Data:        [ 16 samples by 4 sample variables ]:
## tax_table()   Taxonomy Table:     [ 5487 taxa by 8 taxonomic ranks ]:
## taxa are rows
```

```
physeq.bac.species.filt
```

```
## phyloseq-class experiment-level object
## otu_table()   OTU Table:          [ 197 taxa and 15 samples ]:
## sample_data() Sample Data:        [ 15 samples by 4 sample variables ]:
## tax_table()   Taxonomy Table:     [ 197 taxa by 8 taxonomic ranks ]:
## taxa are rows
```

```
# If you choose not to perform filtering, run the following lines:
# physeq.bac.genus.filt <- physeq.bac.genus
# physeq.bac.species.filt <- physeq.bac.species
```

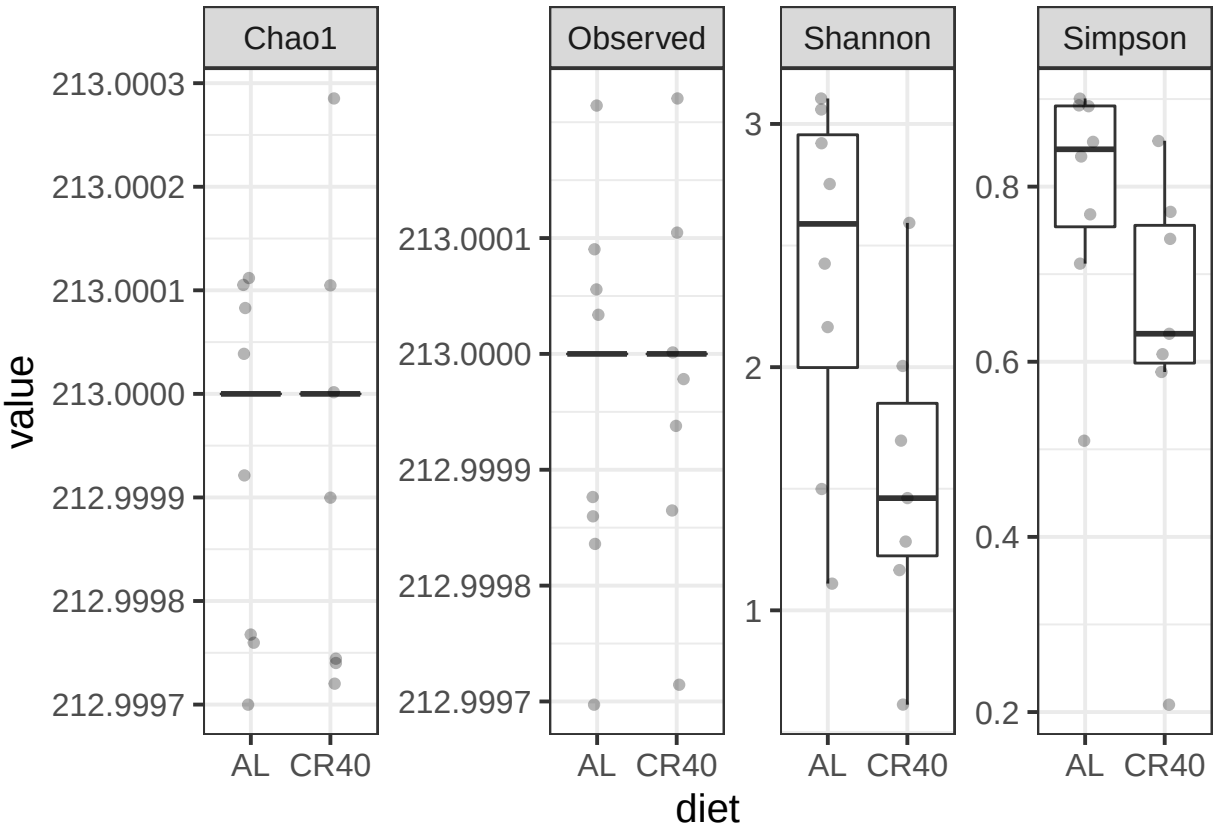
- 16 samples -> 15 samples
- 1463 genera -> 213 genera
- 5487 species -> 197 species

Alpha diversity

Genus

```
alpha.div.genus.df <- estimate_richness(
  physeq.bac.genus.filt, measures=c("Observed", "Chao1", "Shannon", "Simpson"))
```

```
alpha.div.genus.df %>%
  merge(meta.df, by="row.names") %>%
  pivot_longer(c(Observed, Chao1, Shannon, Simpson), names_to="diversity") %>%

  # CHANGEME to show desired comparisons
  ggplot(aes(x=diet, y=value)) +
  geom_boxplot(outlier.shape=NA) +
  geom_jitter(width=0.1, alpha=0.3) +
  facet_wrap(~diversity, ncol=4, scales="free_y")
```

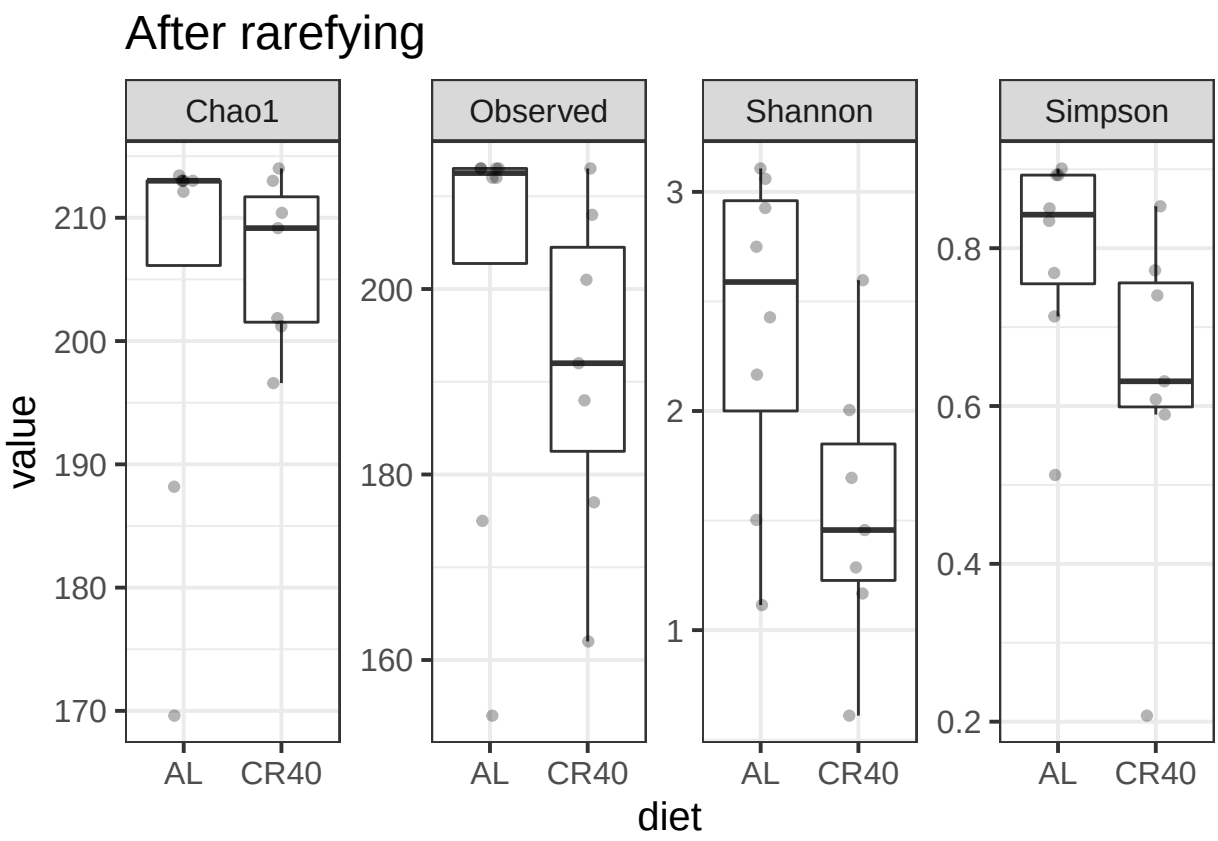


After rarefaction

Alpha diversity is sensitive to sequencing depth, so I recommend performing rarefaction prior to running alpha diversity analysis so that the results aren't confounded by sequencing depth.

```
alpha.div.genus.rarefied.df <- physeq.bac.genus.filt %>%
  rarefy_even_depth(rngseed=101, replace=F, verbose=F) %>%
  estimate_richness(measures=c("Observed", "Chao1", "Shannon", "Simpson"))
```

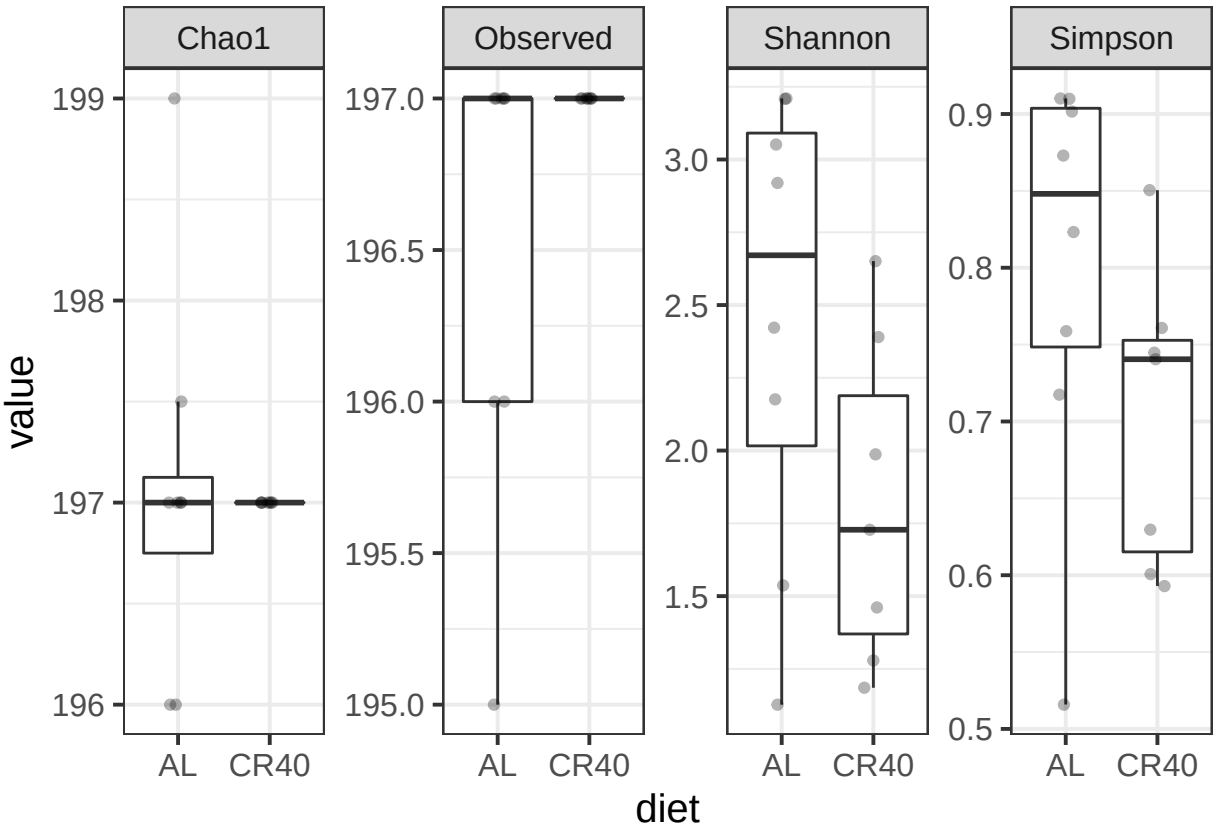
```
alpha.div.genus.rarefied.df %>%
  merge(meta.df, by="row.names") %>%
  pivot_longer(c(Observed, Chao1, Shannon, Simpson), names_to="diversity") %>%

  # CHANGEME to show desired comparisons
  ggplot(aes(x=diet, y=value)) +
  geom_boxplot(outlier.shape=NA) +
  geom_jitter(width=0.1, alpha=0.3) +
  facet_wrap(~diversity, ncol=4, scales="free_y") +
  labs(title="After rarefying")
```



Species

```
alpha.div.species.df <- estimate_richness(
  physeq.bac.species.filt, measures=c("Observed", "Chao1", "Shannon", "Simpson"))
```

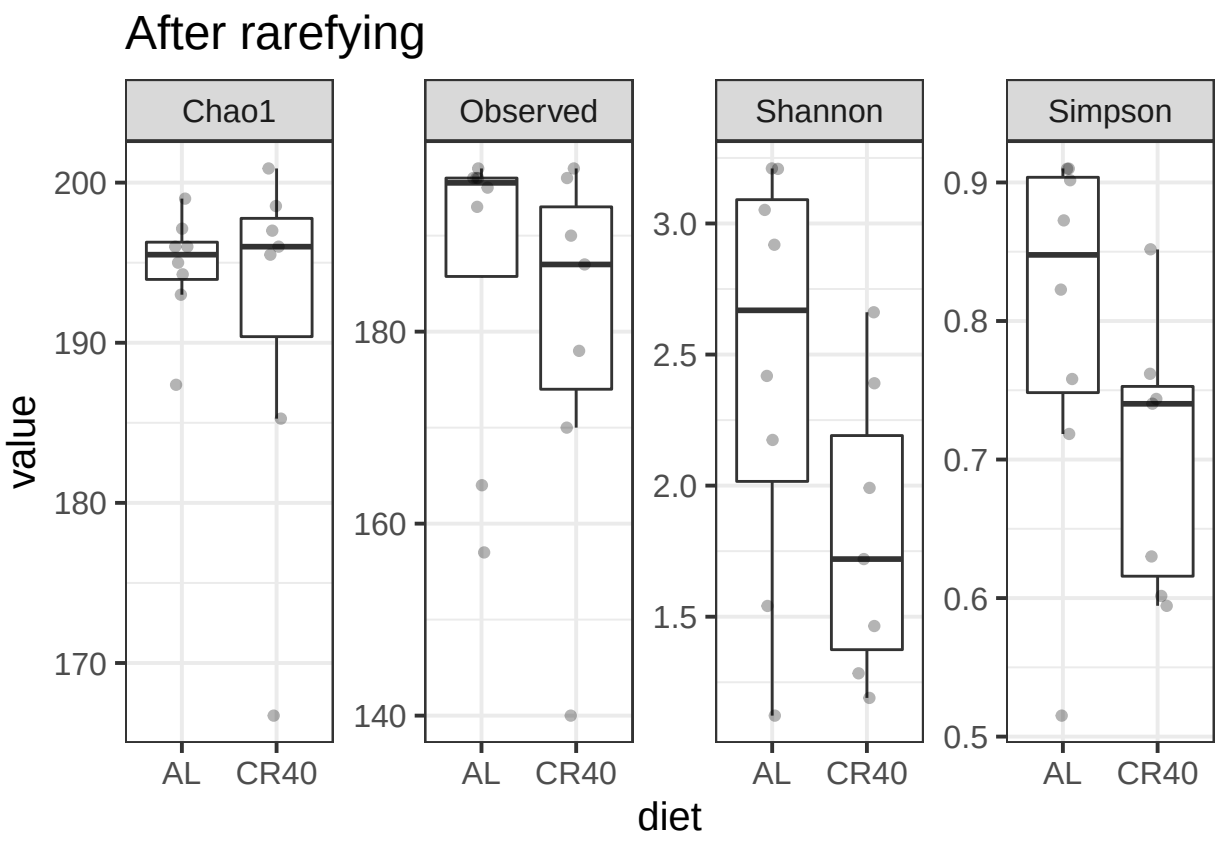
```
alpha.div.species.df %>%
  merge(meta.df, by="row.names") %>%
  pivot_longer(c(Observed, Chao1, Shannon, Simpson), names_to="diversity") %>%

  # CHANGEME to show desired comparisons
  ggplot(aes(x=diet, y=value)) +
  geom_boxplot(outlier.shape=NA) +
  geom_jitter(width=0.1, alpha=0.3) +
  facet_wrap(~diversity, ncol=4, scales="free_y")
```



After rarefaction

```
alpha.div.species.rarefied.df <- physeq.bac.species.filt %>%
  rarefy_even_depth(rngseed=101, replace=F, verbose=F) %>%
  estimate_richness(measures=c("Observed", "Chao1", "Shannon", "Simpson"))
```

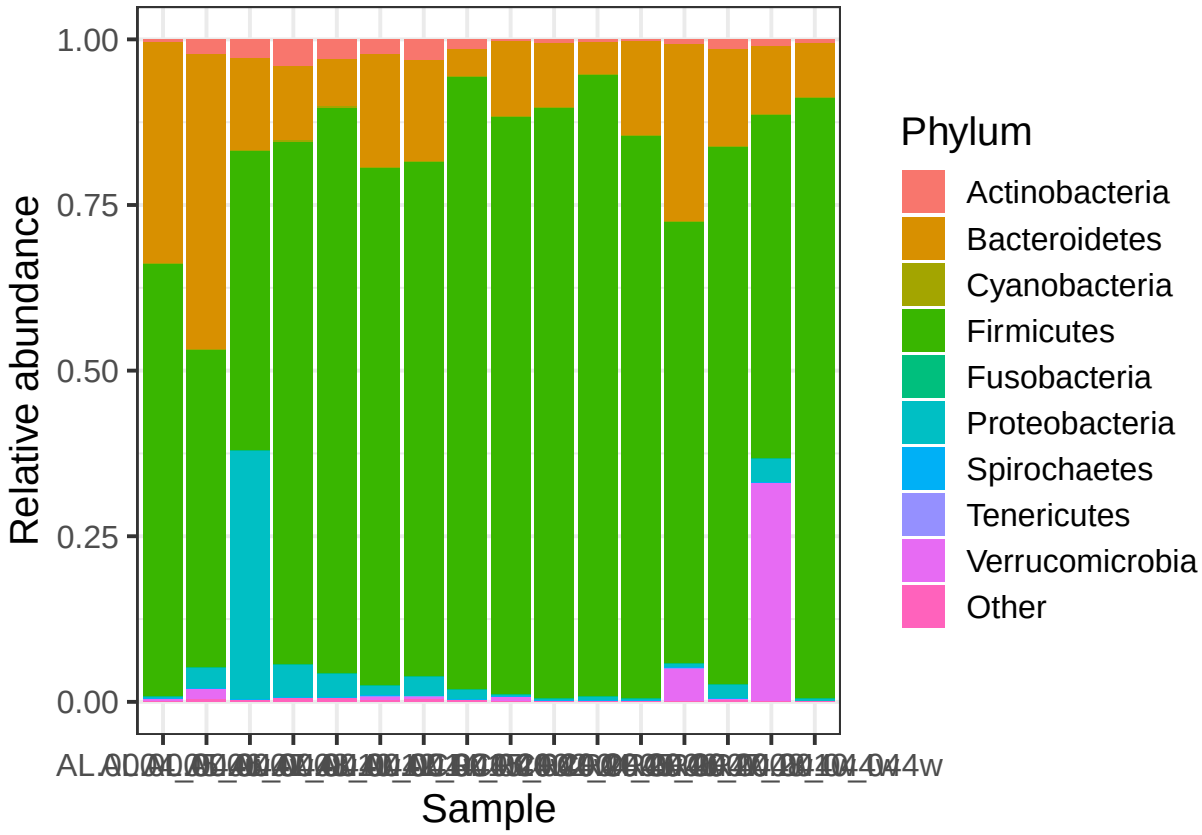
```
alpha.div.species.rarefied.df %>%
  merge(meta.df, by="row.names") %>%
  pivot_longer(c(Observed, Chao1, Shannon, Simpson), names_to="diversity") %>%

  # CHANGEME to show desired comparisons
  ggplot(aes(x=diet, y=value)) +
  geom_boxplot(outlier.shape=NA) +
  geom_jitter(width=0.1, alpha=0.3) +
  facet_wrap(~diversity, ncol=4, scales="free_y") +
  labs(title="After rarefying")
```



Barplots

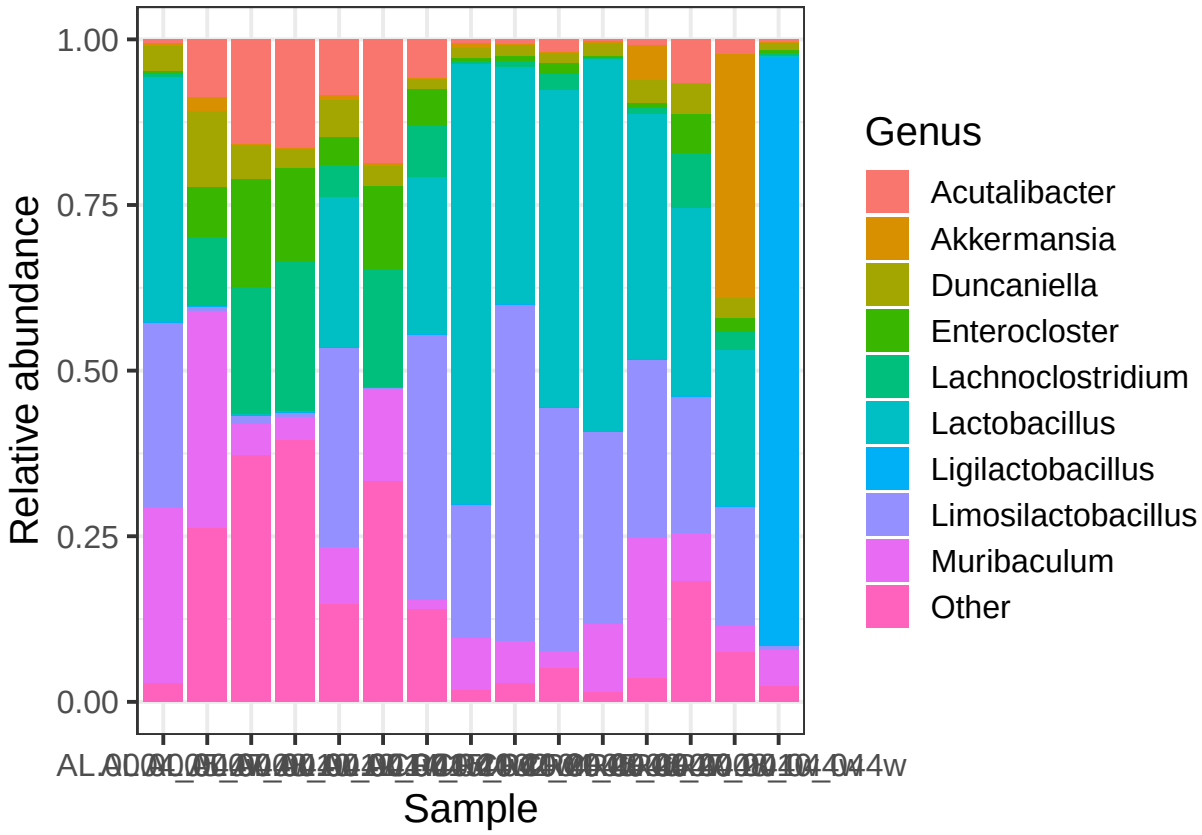
Phylum

```
top.n.phyla <- data.frame(tax_table(physeq.bac.phylum))[
  names(sort(taxa_sums(physeq.bac.phylum), decreasing=T))[1:9], "phylum"]

physeq.bac.phylum %>%

  # compute relative abundance
  transform_sample_counts(function(OTU) OTU/sum(OTU)) %>%

  # convert to df
  psmelt %>%

  # aggregate less common taxa into "Other"
  mutate(agg.phylum=fct_relevel(
    ifelse(phylum %in% top.n.phyla, phylum, "Other"), "Other", after=Inf)) %>%

  # CHANGEME to show desired comparisons
  ggplot(aes(x=Sample, y=Abundance, fill=agg.phylum)) +
  geom_bar(stat="identity") +
  labs(y="Relative abundance", fill="Phylum")
```



Genus

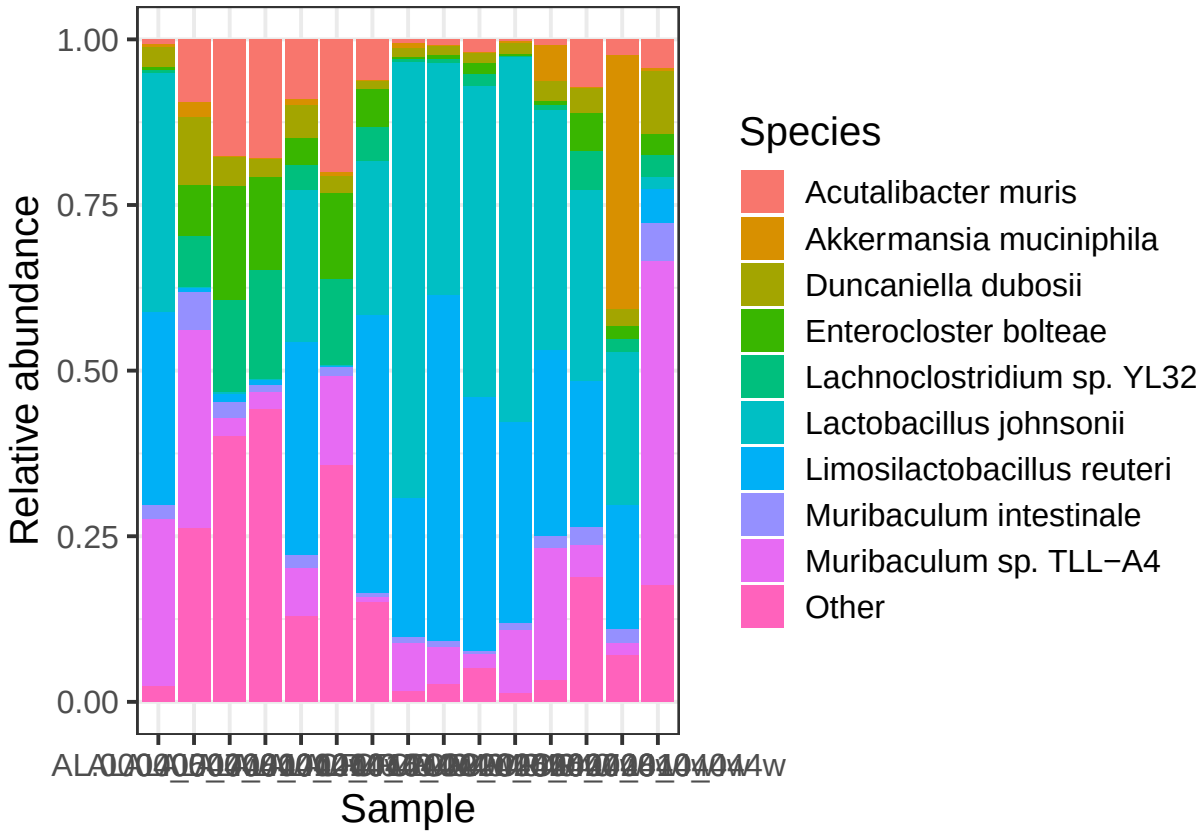
```
top.n.genera <- data.frame(tax_table(physeq.bac.genus.filt))[
  names(sort(taxa_sums(physeq.bac.genus.filt), decreasing=T))[1:9], "genus"]

physeq.bac.genus.filt %>%

  # compute relative abundance
  transform_sample_counts(function(OTU) OTU/sum(OTU)) %>%

  # convert to df
  psmelt %>%

  # aggregate less common taxa into "Other"
  mutate(agg.genus=fct_relevel(
    ifelse(genus %in% top.n.genera, genus, "Other"), "Other", after=Inf)) %>%

  # CHANGEME to show desired comparisons
  ggplot(aes(x=Sample, y=Abundance, fill=agg.genus)) +
  geom_bar(stat="identity") +
  labs(y="Relative abundance", fill="Genus")
```



Species

Use `genus_and_species` annotation to improve the labels on the plot.

```
top.n.species <- data.frame(tax_table(physeq.bac.species.filt))[
  names(sort(taxa_sums(physeq.bac.species.filt), decreasing=T))[1:9], "genus_and_species"]

physeq.bac.species.filt %>%

  # compute relative abundance
  transform_sample_counts(function(OTU) OTU/sum(OTU)) %>%

  # convert to df
  psmelt %>%

  # aggregate less common taxa into "Other"
  mutate(agg.species=fct_relevel(
    ifelse(genus_and_species %in% top.n.species, genus_and_species, "Other"), "Other", after=Inf)) %>%

  # CHANGEME to show desired comparisons
  ggplot(aes(x=Sample, y=Abundance, fill=agg.species)) +
  geom_bar(stat="identity") +
  labs(y="Relative abundance", fill="Species")
```

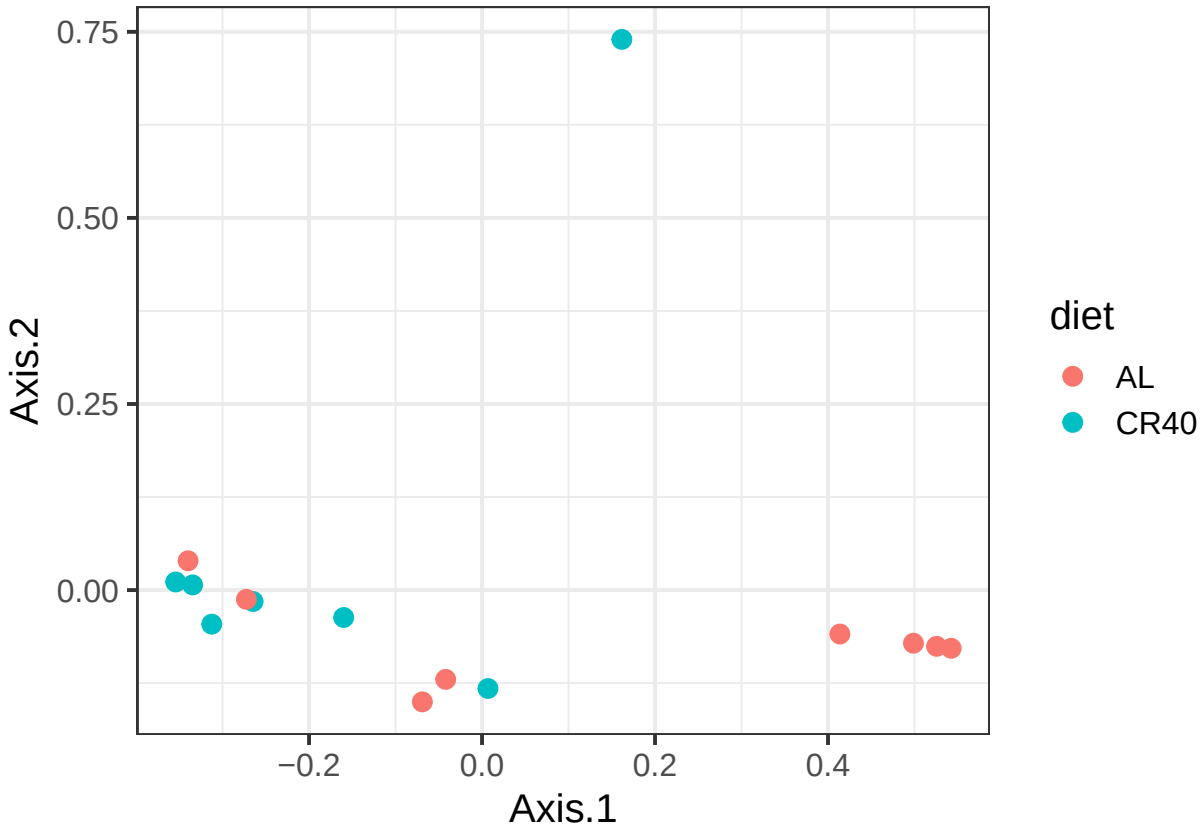


PCoA

Genus

```
pcoa.bac.genus <- physeq.bac.genus.filt %>%

  # compute relative abundance
  transform_sample_counts(function(OTU) OTU/sum(OTU)) %>%

  # perform PCoA using Bray-Curtis distance
  ordinate(method="MDS", distance="bray")

# create coordinates
pcoa.bac.genus.df <- plot_ordination(physeq.bac.genus.filt, pcoa.bac.genus,
                                     type="samples", justDF=T)
```

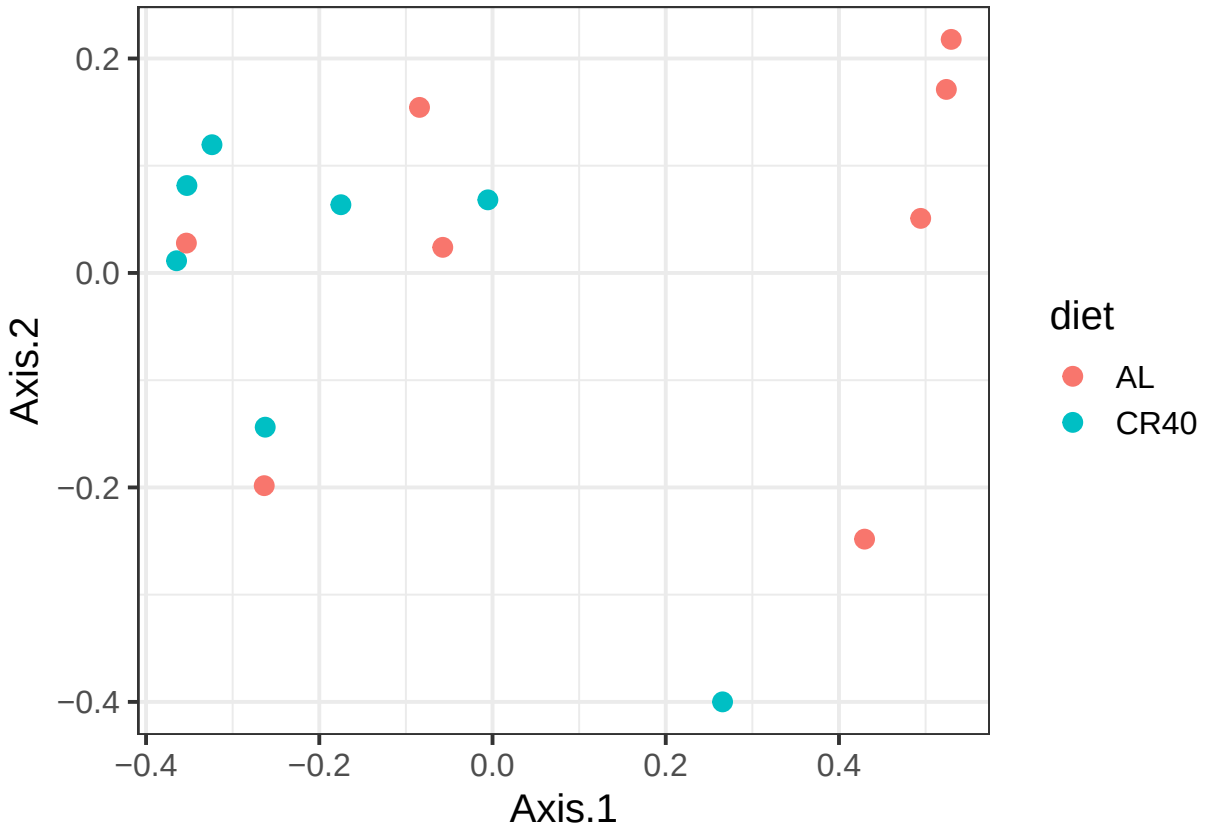
```
# CHANGEME to show desired comparisons
pcoa.bac.genus.df %>%
  ggplot(aes(x=Axis.1, y=Axis.2, color=diet)) +
  geom_point(size=3)
```



Species

```
pcoa.bac.species <- physeq.bac.species.filt %>%

  # compute relative abundance
  transform_sample_counts(function(OTU) OTU/sum(OTU)) %>%

  # perform PCoA using Bray-Curtis distance
  ordinate(method="MDS", distance="bray")

# create coordinates
pcoa.bac.species.df <- plot_ordination(physeq.bac.species.filt, pcoa.bac.species,
                                       type="samples", justDF=T)
```

```
# CHANGEME to show desired comparisons
pcoa.bac.species.df %>%
  ggplot(aes(x=Axis.1, y=Axis.2, color=diet)) +
  geom_point(size=3)
```



DESeq2 to find differential taxons

Genus

```
deseq.genus <- physeq.bac.genus.filt %>%

  # CHANGEME: update the design formula to perform desired comparisons
  phyloseq_to_deseq2(~diet)
```

```
## converting counts to integer mode
## Warning in DESeqDataSet(se, design = design, ignoreRank): some variables in
## design formula are characters, converting to factors
```

```
deseq.genus <- DESeq(deseq.genus)
```

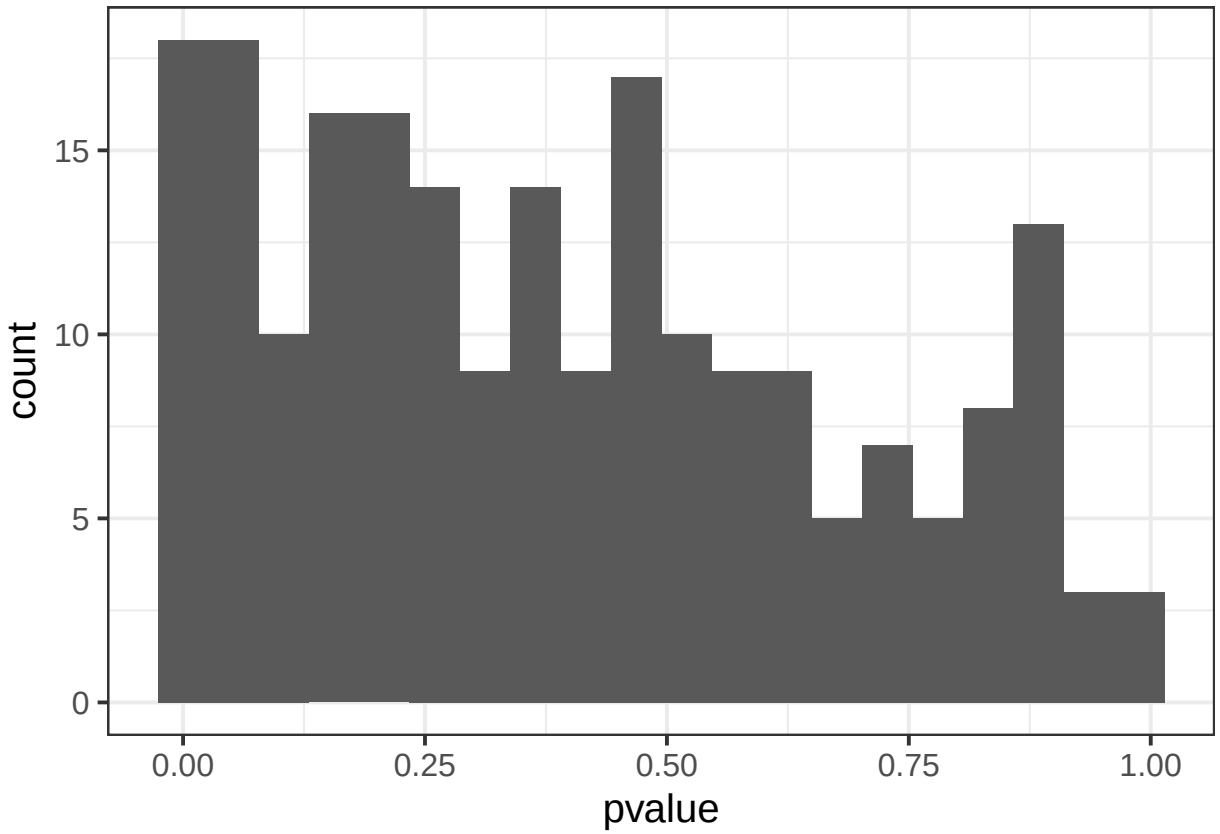
```
## estimating size factors
## estimating dispersions
## gene-wise dispersion estimates
## mean-dispersion relationship
## -- note: fitType='parametric', but the dispersion trend was not well captured by the
##    function: y = a/x + b, and a local regression fit was automatically substituted.
##    specify fitType='local' or 'mean' to avoid this message next time.
## final dispersion estimates
## fitting model and testing
```

```
## -- replacing outliers and refitting for 4 genes
## -- DESeq argument 'minReplicatesForReplace' = 7
## -- original counts are preserved in counts(dds)
```

```
## estimating dispersions
```

```
## fitting model and testing
```

```
results(deseq.genus) %>% as.data.frame %>%
  ggplot(aes(pvalue)) +
  geom_histogram(bins=20)
```



- The shape of this histogram tells you whether to expect any significant results

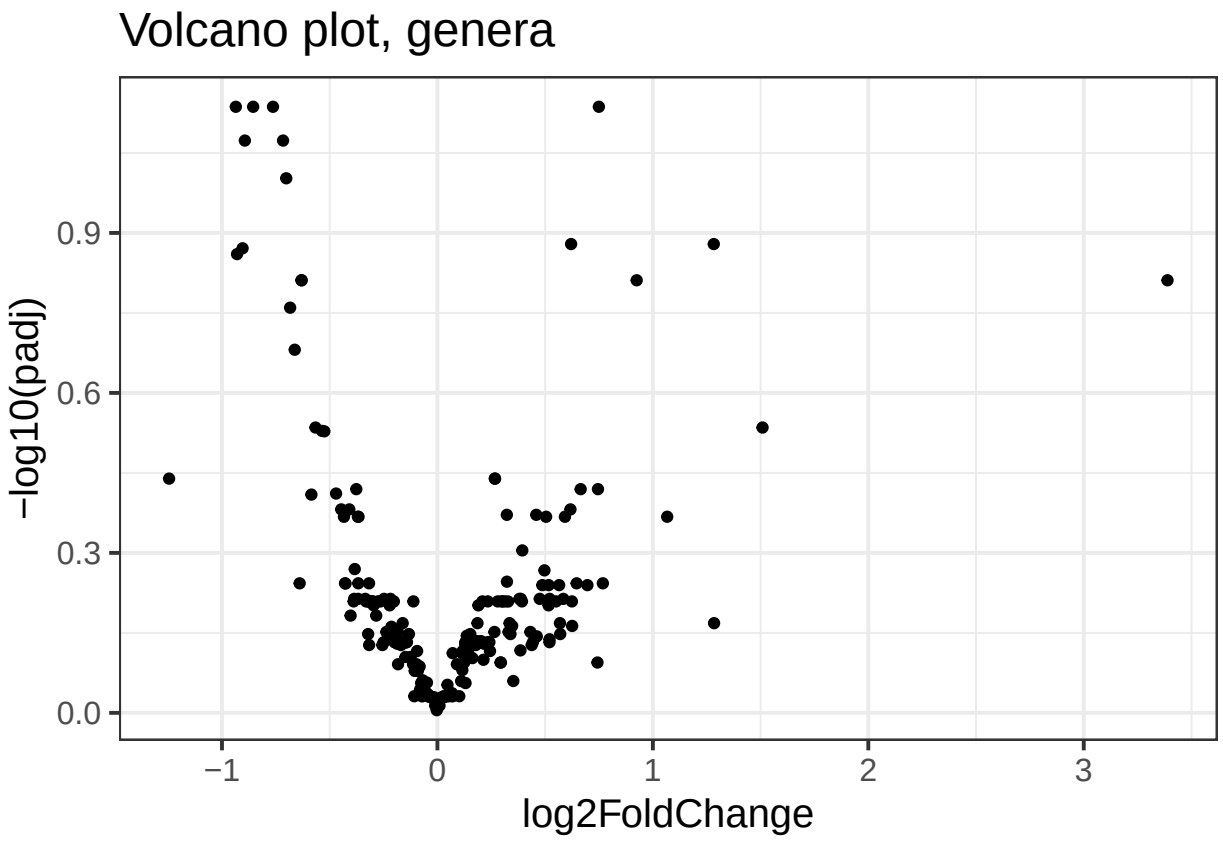
```
results(deseq.genus) %>% as.data.frame %>%
  dplyr::filter(padj < 0.1)
```

```
##           baseMean log2FoldChange     lfcSE      stat       pvalue       padj
## 39488    393.97495     -0.8936661 0.2941429 -3.038204 0.0023799284 0.08448746
## 2763056 1450.57113     -0.7016046 0.2385345 -2.941313 0.0032682398 0.09944787
## 35701     17.61384     -0.7630718 0.2356969 -3.237514 0.0012057621 0.07301969
## 2583452   46.21434     -0.7164464 0.2347089 -3.052489 0.0022695182 0.08448746
## 365349    23.02017      0.7494517 0.2243861  3.340009 0.0008377557 0.07301969
## 128785    15.30414     -0.8552258 0.2431065 -3.517907 0.0004349655 0.07301969
## 81412     13.61889     -0.9356860 0.2923441 -3.200633 0.0013712618 0.07301969
```

- 7 results with adjusted p-value < 0.1
- The adjusted p-value threshold is somewhat arbitrary

```
results(deseq.genus) %>% as.data.frame %>%
  ggplot(aes(x=log2FoldChange, y=-log10(padj))) +
```

```
  geom_point() +
  labs(title="Volcano plot, genera")
```



Species

```
deseq.species <- physeq.bac.species.filt %>%

  # CHANGEME: update the design formula to perform desired comparisons
  phyloseq_to_deseq2(~diet)
```

```
## converting counts to integer mode

## Warning in DESeqDataSet(se, design = design, ignoreRank): some variables in
## design formula are characters, converting to factors
```

```
deseq.species <- DESeq(deseq.species)
```

```
## estimating size factors

## estimating dispersions

## gene-wise dispersion estimates

## mean-dispersion relationship

## -- note: fitType='parametric', but the dispersion trend was not well captured by the
##    function: y = a/x + b, and a local regression fit was automatically substituted.
##    specify fitType='local' or 'mean' to avoid this message next time.

## final dispersion estimates
```

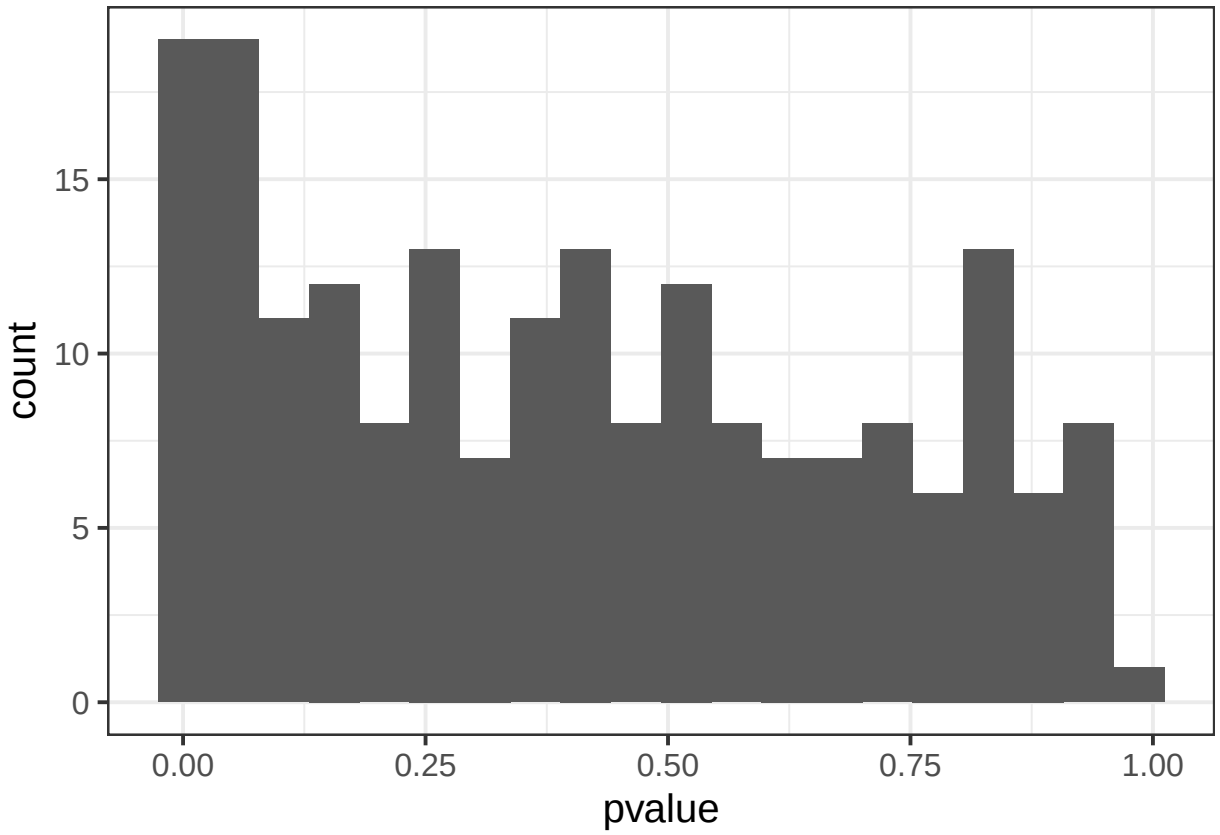
```
## fitting model and testing
```

```
## -- replacing outliers and refitting for 2 genes
## -- DESeq argument 'minReplicatesForReplace' = 7
## -- original counts are preserved in counts(dds)
```

```
## estimating dispersions
```

```
## fitting model and testing
```

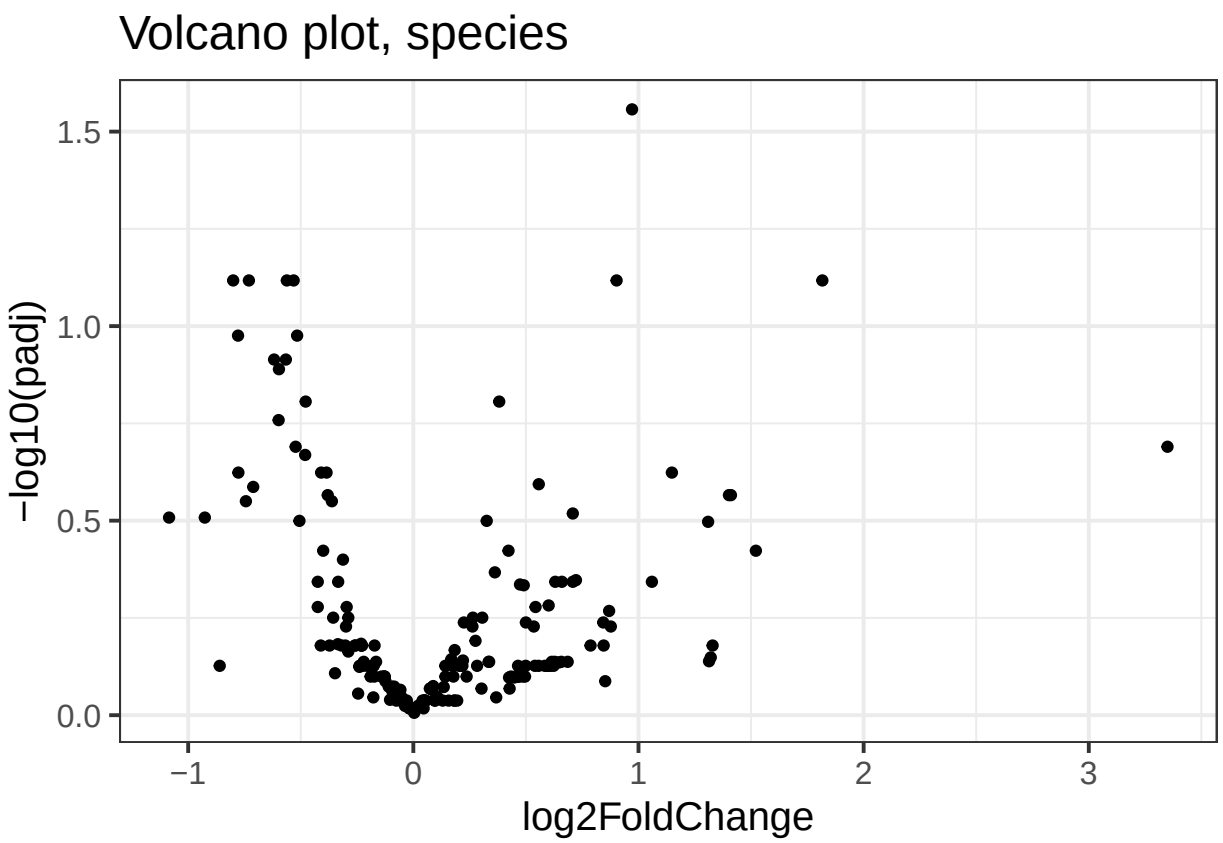
```
results(deseq.species) %>% as.data.frame %>%
  ggplot(aes(pvalue)) +
  geom_histogram(bins=20)
```




```
results(deseq.species) %>% as.data.frame %>%
  dplyr::filter(padj < 0.1)
```

```
##          baseMean log2FoldChange     lfcSE      stat       pvalue       padj
## 1912897 679.75388      0.9713758 0.2551635  3.806877 0.0001407329 0.02772438
## 39488   375.83986     -0.7303005 0.2433224 -3.001370 0.0026876759 0.07631305
## 351091  386.04652     -0.5315480 0.1770845 -3.001662 0.0026850977 0.07631305
## 1584     36.09639      0.9027695 0.2943759  3.066723 0.0021641909 0.07631305
## 414771   93.79130      1.8166514 0.5773366  3.146607 0.0016517677 0.07631305
## 1685     72.10095     -0.5618419 0.1873638 -2.998667 0.0027116313 0.07631305
## 128785   13.34972     -0.8000896 0.2445831 -3.271239 0.0010707740 0.07631305
```

```
results(deseq.species) %>% as.data.frame %>%
  ggplot(aes(x=log2FoldChange, y=-log10(padj))) +
  geom_point() +
  labs(title="Volcano plot, species")
```



Save for reproducibility

```
Sys.Date()
```

```
## [1] "2021-10-12"
```

```
sessionInfo()
```

```
## R version 4.1.0 (2021-05-18)
## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS Big Sur 10.16
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/4.1/Resources/lib/libRblas.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.1/Resources/lib/libRlapack.dylib
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] parallel  stats4    stats     graphics  grDevices utils     datasets
## [8] methods   base
##
## other attached packages:
##  [1] speedyseq_0.5.3.9018        DESeq2_1.32.0
##  [3] SummarizedExperiment_1.22.0 Biobase_2.52.0
##  [5] MatrixGenerics_1.4.2        matrixStats_0.61.0
```

```
##  [7] GenomicRanges_1.44.0        GenomeInfoDb_1.28.1
##  [9] IRanges_2.26.0              S4Vectors_0.30.0
## [11] BiocGenerics_0.38.0         phyloseq_1.36.0
## [13] forcats_0.5.1               stringr_1.4.0
## [15] dplyr_1.0.7                 purrr_0.3.4
## [17] readr_2.0.1                 tidyr_1.1.4
## [19] tibble_3.1.5                ggplot2_3.3.5
## [21] tidyverse_1.3.1
##
## loaded via a namespace (and not attached):
##   [1] colorspace_2.0-2       ellipsis_0.3.2         XVector_0.32.0
##   [4] fs_1.5.0               rstudioapi_0.13        farver_2.1.0
##   [7] bit64_4.0.5            AnnotationDbi_1.54.1   fansi_0.5.0
##  [10] lubridate_1.7.10       xml2_1.3.2             codetools_0.2-18
##  [13] splines_4.1.0          cachem_1.0.5           geneplotter_1.70.0
##  [16] knitr_1.36             ade4_1.7-17            jsonlite_1.7.2
##  [19] broom_0.7.9            annotate_1.70.0        cluster_2.1.2
##  [22] dbplyr_2.1.1           png_0.1-7              compiler_4.1.0
##  [25] httr_1.4.2             backports_1.2.1        assertthat_0.2.1
##  [28] Matrix_1.3-4           fastmap_1.1.0          cli_3.0.1
##  [31] htmltools_0.5.2        tools_4.1.0            igraph_1.2.6
##  [34] gtable_0.3.0           glue_1.4.2             GenomeInfoDbData_1.2.6
##  [37] reshape2_1.4.4         Rcpp_1.0.7             cellranger_1.1.0
##  [40] vctrs_0.3.8            Biostrings_2.60.2      rhdf5filters_1.4.0
##  [43] multtest_2.48.0        ape_5.5                nlme_3.1-152
##  [46] iterators_1.0.13       xfun_0.26              rvest_1.0.1
##  [49] lifecycle_1.0.1        XML_3.99-0.7           zlibbioc_1.38.0
##  [52] MASS_7.3-54            scales_1.1.1           hms_1.1.0
##  [55] biomformat_1.20.0      rhdf5_2.36.0           RColorBrewer_1.1-2
##  [58] yaml_2.2.1             memoise_2.0.0          stringi_1.7.5
##  [61] RSQLite_2.2.7          highr_0.9              genefilter_1.74.0
##  [64] foreach_1.5.1          permute_0.9-5          BiocParallel_1.26.1
##  [67] rlang_0.4.11           pkgconfig_2.0.3        bitops_1.0-7
##  [70] evaluate_0.14          lattice_0.20-44        Rhdf5lib_1.14.2
##  [73] labeling_0.4.2         bit_4.0.4              tidyselect_1.1.1
##  [76] plyr_1.8.6             magrittr_2.0.1         R6_2.5.1
##  [79] generics_0.1.0         DelayedArray_0.18.0    DBI_1.1.1
##  [82] pillar_1.6.3           haven_2.4.3            withr_2.4.2
##  [85] mgcv_1.8-36            KEGGREST_1.32.0        survival_3.2-12
##  [88] RCurl_1.98-1.4         modelr_0.1.8           crayon_1.4.1
##  [91] utf8_1.2.2             tzdb_0.1.2             rmarkdown_2.11
##  [94] locfit_1.5-9.4         grid_4.1.0             readxl_1.3.1
##  [97] data.table_1.14.2      blob_1.2.2             vegan_2.5-7
## [100] reprex_2.0.1           digest_0.6.28          xtable_1.8-4
## [103] munsell_0.5.0
```